PATENT APPLICATION
Navy Case No. **84,517**

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

APPLICATION FOR LETTERS PATENT

TO ALL WHOM IT MAY CONCERN:

BE IT KNOWN THAT **Sean J. Hart and Alexander V. Terray** who are citizens of the United States of America, and are residents of, Alexandria, VA and Alexandria, VA , invented certain new and useful improvements in **"LASER OPTICAL SEPARATOR AND METHOD FOR SEPARATING COLLOIDAL SUSPENSIONS"** of which the following is a specification:

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LASER OPTICAL SEPARATOR AND METHOD FOR SEPARATING COLLOIDAL SUSPENSIONS

BACKGROUND OF THE INVENTION

1. Field of the Invention

This invention deals generally with optical separation of microscopic particles separation for the purposes of identification. Specifically the particles to be separated are between 0.1-30 μm in diameter. The invention is to be used as a fundamental tool of chemical and biological colloidal analysis.

2. Description of the Related Prior Art

The invention of the laser has made possible many new areas of research and technology. Unique optical properties allowing a laser to be highly focused have made detailed studies of radiation pressure possible. Most important is the laser's ability to focus down to a tiny spot size, resulting in a large photon density. This large number of photons translates into a significant amount of radiation force applied to a particle in the beam path. Radiation pressure has been used to trap and direct particles caught in the focus of a laser beam. Manipulation of the beam focus and beam position can be used to move particles into desired positions and configurations. (See M. M. Koshioka, K. Sasaki, N. Kitamura, H. Masuhara, *Chemistry Letters*, 1479-1482, 1990; M. M. Koshioka, K. Sasaki, N. Kitamura, H. Masuhara, *Optics Letters*, 16, 19, 1463, 1991; A. Ashkin, J.M. Dziedzic, and T. Yamane, *Nature*, 330, 769, 1987) The types of objects that

have been optically trapped include glass and polymer spheres, viruses, bacteria, and biological cells. (See A. Ashkin, J.M. Dziedzic, and T. Yamane, *Nature*, 330, 769, 1987; A. Ashkin, J.M. Dziedzic, *Science*, 245, 1517, 1987) Recently, size-based separation of particles flowing in a fluid opposite to the direction of laser propagation has been achieved. (See T. Imasaka, Y. Kawabata, T. Kaneta, and Y. Ishidzu, *Anal. Chem.* 1995, 67(11), 1763-1765)

In recent years, a technique has been developed, termed optical chromatography, which was employed for the laser separation of differently sized particles in the 1-10 micron range. (See T. Imasaka, Y. Kawabata, T. Kaneta, and Y. Ishidzu, *Anal. Chem.* 1995, 67(11), 1763-1765; T. Kaneta, Y. Ishidzu, N. Mishima, and T. Imasaka, *Anal. Chem.*, 69, 2701-2710, 1997; J. Makihara, T. Kaneta, T. Imasaka, *Talanta*, 48, 551-557, 1999) When particles in a liquid flowing within a capillary encounter a laser beam propagating in the opposite direction the particles are subjected to optical pressure near the beam focal point (region of highest photon density) intense enough to impart momentum sufficient to overcome fluid drag forces. The result is that particles in the fluid become trapped and move against the fluid flow until the beam diverges and the photon density decreases. The particles remain stationary when the optical pressure equals the force exerted on the particles by the liquid flow (Stoke's force).

For a sphere of refractive index n_2 in a medium of lower refractive index, n_1, the force due to optical pressure of the laser, $F_{optical\_pressure}$, is given by equation 1:

$$F_{optical\_pressure} = \frac{2n_1 P}{c}\left(\frac{a}{\omega}\right)^2 Q^* \qquad (1),$$

Where P is the power of the laser, c is the speed of light, a is the sphere radius, ω is the beam radius, and Q^* is the conversion efficiency of optical radiation pressure to Newtonian force on the particle. (See A. Ashkin, *Biophys. J.*, 61, 569-582, 1992) The term (n_1P/c) defines the incident momentum per second in a medium of refractive index n_1. The dimensionless parameter, Q^* defines the conversion efficiency of optical pressure transfer arising from light reflection and refraction based upon geometrical considerations and is calculated using the Fresnel reflection and transmission coefficients, which in turn depend upon n_2, the refractive index of the particle.

Separation in a liquid flow is measured by the distance particles travel away from the focal point against the fluid flow. This distance traveled is the optical retention distance (See T. Kaneta, Y. Ishidzu, N. Mishima, and T. Imasaka, *Anal. Chem.*, 69, 2701-2710, 1997), z: the point at which the optical pressure equals the force exerted on the spheres by the liquid molecules and is defined, according to Equation 2:

$$z = \frac{\pi\omega_0^2}{\lambda}\sqrt{\frac{n_1 PQa}{3\pi\eta v c\omega_0} - 1} \qquad (2),$$

where the power of the TEM_{00} mode laser, P, c is the speed of light, a is the sphere radius, ϖ is the beam radius at the focal point, λ is the wavelength of light, v is the velocity of the particle in the water flow, and η is the viscosity of water. The refractive index of the particle is used in the calculation of the efficiency of optical pressure transfer, Q.

Optical pressure has been used extensively in research and industry for biological size-based micromanipulation. The chemical effect on optical pressure in bacteria has been observed: small chemical differences in the surface coatings have been shown to result in large force differentials on different strains of the same species of non-pathogenic bacteria. (See C. S. Buer, K. T. Gahagan, G. A. Swartzlander, P. J. Weathers, *Journal Of Industrial Microbiology & Biotechnology*, 21: (4-5) 233-236 1998) However, the theoretical chemical dependence, development, and use of optical pressure chemical differentials for separation have not yet been demonstrated. Free from the requirements of chemical or immunological systems, a technique based upon optical separation and detection alone will outperform the current levels of performance achieved using other methods. Systems based upon immunology require significant time and cost outlays to develop antibodies for new BW agents. (See F. S. Ligler, G. P. Anderson, P. T. Davidson, R. J. Foch, J. T. Ives, K. D. King, G. Page, D. A. Stenger, and J. P. Whelan, *Environ. Sci. Technol.*, 32, 2461-2466, 1998) Furthermore, with these techniques, the detection of modified and/or unknown species in real time is not possible. Other techniques based upon DNA analysis, while very accurate, have problems including long

analysis times, high cost and complexity, and delicate instrumentation which is unlikely to be reduced in size significantly. Other methods of BW agent detection include fluorescence detection of aerosols. (See N. F. Fell, et al., SPIE Vol. 3533, 52, **1999**) Such techniques are limited in their ability to detect BW agents, as intrinsic fluorescence is derived from three amino acids (with similar excitation and emission spectra) common to many biological particles. Detection based upon fluorescence alone is not likely to enable characterization of closely related species.

Field flow fractionation (FFF) is used for the separation of particulate materials based upon their size, mass, density, charge, or other physical properties. (See T. Chianea, N E. Assidjo, P. J. P. Cardot, *Talanta*, 51, 835-847, 2000; K. G. Wahlund, and J. C. Giddings, *Anal. Chem.*, 59. 1332-1339, 1987) The most basic variant of FFF uses a flow field to carry particulates in flow down a thin channel. These particles are then subjected to a force (gravitional, flow, electrical, or other) that causes them to accumulate differentially at the wall edge of the laminar flow field. Particles least affected by the applied field will travel down the channel and exit sooner than those that are more effected by the applied field. Recently, strains of *E.coli* have been separated using a variation of FFF based upon the presence or absence of fimbriae. (P. Reschiglian, A. Zattoni, B. Roda, S. Casolari, M. H. Moon, J. Lee, J. Jung, K. Rodman, *Anal. Chem.*, 74, 4895-4904, 2002)

Flow cytometry is a technique used for characterizing cell populations wherein a sheath flow fluidic system hydrodynamically focuses the cells into a line. (See A. Gilman-Sachs, *Anal. Chem.*, 66, 13, 1994; J. W. Hofstrat, W. J. M., van Zeijl, J. C. H.,

Peeters, and L. Peperzak, *Anal. Chim. Acta.*, 290, 135-145, 1994) Once in a line the stream containing the cells is interrogated by one or more laser beams of differing wavelength. Laser light scattering and laser induced fluorescence (for dye labeled particles or cells) measurements are made of the passing samples. From these, many parameters can be determined including size, volume, granularity, and biochemical properties using dye labeled cell surface antigens. In a cell sorting flow cytometer, after the optical measurements, the sheath flow is vibrated at a high velocity creating tiny droplets, which ideally contain only one cell. Depending on the cell type determined by the laser measurements, a charge is applied to the droplets. When these charged droplets pass between two charged plates they are deflected and can be collected, resulting in a specifically directed separation.

While the above techniques enable separation, they suffer limitations that the current invention alleviates. Discrimination is not inherently based upon intrinsic chemical composition when using field flow fractionation. This limits the technique to essentially size based separation which are not as universally important as biochemical specificity when dealing with microbiological samples. With respect to flow cytometry, sorting can be achieved based upon physical properties and biochemical information derived from specific fluorescent probes. While being a powerful bioanalytical technique, flow cytometry suffers from the cost and complexity of the fluid system and the multiple color lasers required to excite fluorescence in dye labeled biochemical markers. More

importantly, much biochemical specificity and identification are achieved through the use of bioprobes, which by definition require prior knowledge for successful application. A method such as optical laser separation which relies on the intrinsic characteristics of the biological species should prove more versatile and capable.

SUMMARY OF THE INVENTION

An objective of this invention is to provide a particle separator using laser optical pressure to separate microscopic particles .01-30 μm in diameter.

The invention accomplishes separation by using a balance of forces with light interaction causing momentum transfer with particles of interest generating optical pressure and viscous drag force of an opposing fluid flow.

The invention uses a laser beam to cause particles of larger effective size or higher refractive index to experience a greater optical force and thus be separated from particles of smaller size or refractive index.

The possibility of separating chemically different particles offers important new possibilities for analysis and possible purified collection of colloidal samples such as organic particulates, inorganic particles (glass and metal particles), and biological species such as cells, bacteria, and viruses. Differentiation of biological samples such as bacteria is based upon chemical differences in their capsules. Polysaccharides, lectins, lipoteichoic acids, and proteins are some of the biomolecules present in various bacterial species and strains. It is well known that there exists a substantial range of refractive indices in bacterial and viral samples due to their different chemical compositions. (See J. B. Bateman, J. Wagman, and E. L. Carstensen, Kolloid-Zeitschrift und Zeitschrift fur Polymere, Band 208, Heft 1, 1965) The ability to separate biological species based upon physical and chemical properties using only light interaction with samples in a simple fluid flow is new and has great potential benefits when applied to bio-warfare detection

and biomedical analysis. Not only are samples physically separable using light, but from their position in the separation field one can determine their refractive index. Thus, from a predicted location one may identify specific entities in an unknown mixture.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 is an illustration of the basic experimental setup of the invention illustrating the retention distance, Z, defined as the distance from the focal point a particle has traveled upstream against the liquid flow

Figure 2 is the general flowcell design of the invention showing the direction of liquid flow and the counter propagating focused laser beam entering the flowcell

Figure 3a is a side view drawing of the glass "L" shaped flowcell. and Figure 3b is a top down view of the glass "L" shaped flowcell.

Figure 4a is a end on view drawing of the glass "L" shaped flowcell with side window for alignment and Figure 4b is top down view of the "L" shaped flowcell.

Figure 5a is an image of the photomask used to produce the master mold of the laser flowcell with added components and Figure 5b is a side view of the assembled PDMS (poly-dimethylsiloxane) flowcell

Figure 6a is a top down view of the PDMS fluidic connection platform showing overlap with the PDMS fluidic network, and Figure 6b is a side view of the PDMS fluidic connection platform.

Figure 7 is a diagram of the optical separation instrument of the invention.

Figure 8 is a microscope image depicting the laser beam focal point focused into the glass flowcell. The approximate focal point, denoted by the arrow, was imaged in a fluorescent solution of safranin O. Small contaminant particles can be seen scattering in the beam

Figure 9a shows the laser beam focused directly into the PDMS flowcell containing water through the PDMS wall and Figure 9b shows the laser beam focal point focused into the PDMS flowcell containing water with the incorporated glass window.

Figure 10a shows data of the optical separation of 2.0 mm PS and PMMA beads with pure PMMA injected and Figure 10b shows data of the optical separation with pure PS injected, and Figure 10c shows data of the optical separation with a mixture of PS (I) and PMMA (II) beads injected. The field of views are slightly different as the objective was moved to center the images. However, the red line is a reference point fixed to a feature

in the glass. The liquid flow travels from right to left, and the laser propagates from left to right

Figure 11 is a summary of the mean retention distances for the PS and PMMA optical separation experiments

DETAILED DESCRIPTION OF THE INVENTION

This patent disclosure describes the instrumentation and methodology for the laser separation of particles using light-induced pressure. The invention employs a phenomenon called optical pressure to separate particles of varying refractive indices. Optical pressure occurs when photons interact with microscopic particles imparting a fraction of their momentum (photon momentum) when they scatter at the surface or refract through the particle. This effect is significant when using lasers due to the extremely large number of photons available. Particles experience a radiation pressure force in the direction of the laser propagation and towards regions of high intensity, i.e. the focal point. The magnitude of the optical pressure depends on chemical composition (refractive index) of the particle, and its size. (See A. Ashkin, *Biophys. J.*, 61, 569-582, 1992; T. Kaneta, Y. Ishidzu, N. Mishima, and T. Imasaka, *Anal. Chem.*, 69, 2701-2710, **1997**; A. Ashkin, J.M. Dziedzic, J.E. Bjorkholm, S. Chu, *Optics Letters*, 11, 5, 288, **1986**) While the magnitude of optical pressure is due several properties including size, shape, and refractive index, laser trapping and separation research has focused primarily on the size dependence and the development of laser micro-manipulation techniques. (See K. Taguchi, H, Ueno, Y. Hiramatsu, and M. Ikeda, Electronics Letters, 33, 5, 1997; C. Mio, T, Gong, A. Terray, and D. W. M. Marr, "*Review of Scientific Instruments*", 71, 5, 2196-2200, 2000)

The hitherto neglected refractive index dependence of radiation pressure has been theoretically demonstrated to allow separation of chemically different materials. (See S.

J. Hart, NRL Memorandum Report, NRL/MR/6110--01-8555, July, 9, 2001) Although refractive index affects the optical pressure force acting on a particle, it is its chemical composition that leads to its unique refractive index. A fluidic devices designed to be used with a loosely focused laser beam propagating against a liquid flow have been developed to separate chemically different particles. Particles of larger refractive index or size will experience a greater optical force and will have a greater retention distance (i.e. will be optically pushed farther upstream from the laser focal point against the liquid flow).

A pictorial representation of the experiment and the retention distance is shown in Figure 1. The laser 100 emits a beam 110 which is focused with a lens 120 to a focal point 130 in a flowcell 140. Particles experiencing laser pressure move in the direction of laser propagation, away from the focal point against the liquid flow 150. They come to rest when the optical pressure force on the particles equals the force due to the liquid flow.

The fluidic devices and optical components necessary for differential optical pressure retention of chemically different particles have been reduced to practice and tested. Micron-sized spheres of identical size, but of different chemical composition and thus refractive indices, moving in a liquid flow have been separated by an opposing laser beam.

Experimental

Fluidic devices for optical separation have been constructed using glass and poly(dimethylsiloxane) (PDMS). The flowcells were designed and constructed to be visibly transparent, over a long linear path (5 - 10cm), contain non-curved surfaces, and have a high-quality optical window for laser access.

Figure 2 is a generic drawing exemplifying the simplest fluid path design in the flowcell with the laser beam 210 focused by a lens 200 into the linear channel through a window 220. Flowing liquid carrier stream and injected particulate samples are introduced into the flowcell via tubing 230 at the flowcell entrance 240, experience laser pressure just after the focal point 250 in the linear channel. The laser beam enters the flowcell through the quartz window at "L" junction of the flowcell 260, and fluid exits the flowcell further downstream 270 through tubing 280.

Glass flowcells

These flowcells were assembled from rectangular glass flats (borosilicate glass) and square UV windows (fused silica) using UV-cured epoxies of different viscosities to cement the optical element together. Tubing (0.02" i.d.) was fixed at the inlets and outlets of the glass flowcells also using UV-cured epoxy. The typical dimensions were 1 mm high, 1 or 2 mm wide and 75 mm long resulting in volumes of 75 μL for 1 mm width and 150 μL for 2 mm width.

Figures 3a and 3b show the simplest design of the glass flowcell; the numbering of the elements in both views (a and b) are consistent. The optical flats, bottom 340, left

330, right 320, and the top 310 were assembled as a sandwich creating a space 360 for the fluid pathway 360. The left glass flat 330 was moved backwards to create an "L" shaped geometry as seen in Figure 3b. The unsealed pathways 360 were covered and sealed with the square fused silica optical window 300. In Figure 3b, the glass flat 340 is not visible because the other components are on top. The liquid and samples enter the flowcell via tubing 350 and exit via tubing 320, connected by the fluid pathway 360. A critical requirement for optical separation when using a pressure based fluid delivery system is alignment of the laser beam and the fluid channel. Since the velocity profile of the fluid channel is parabolic, any misalignment of the laser beam and the tip of the fluid parabola may result in non-symmetric flow patterns rather than separation.

Figures 4a and 4b show the flowcell design which incorporates a side window allowing visual access along the fluidic channel for laser - channel alignment; the numbering of the elements in both views (a and b) are consistent. The optical flats, top 410, left 430, right 420, and the bottom 440 were assembled as a sandwich creating a space 460 for the fluid pathway 460. The left glass flat 430 was moved backwards to create an "L" shaped geometry as seen in Figure 4b. Turning 430 on its edge permits optical viewing of the laser beam inside the flowcell, thus greatly facilitating alignment. The unsealed pathways 460 were covered and sealed with the square fused silica optical window 400. In Figure 4b, the glass flat 440 is not visible because the other components are on top. The liquid and samples enter the flowcell via tubing 450 and exit via tubing 420, connected by the fluid pathway 460.

PDMS flowcells

Flowcells of this type were manufactured using soft lithography techniques. (See D. C. Duffy, J. C. McDonald, O. J. A. Schueller, G. M. Whitesides, Anal. Chem., 70, 4974-4984, 1998) This involves the creation of a silicon substrate master for PDMS replica molding and is created using standard photolithographic methods. A 1 to 2 mm thick layer of SU-8 photoresist (MicroChem Corp.) was patterned via contact mode photolithography using a high-resolution transparency mask. This patterned layer was further developed to create the master mold.

Figure 5a shows the basic design of the PDMS flowcell in a top down view. The volume was comparable to the glass flowcell: for a width of 1 mm, a total length of 4 cm, and the height was approximately 1.8 mm, the total volume was only 76 μL. The flowcell is used in the following manner: The fluid and particles enter the flowcell via the inlet 500 travel along the linear separation channel 510. The laser 560 enters the flowcell at location 520 and fluid exits the flowcell at the outlet 570. The image in Figure 5a, is the actual photomask which was used to create the master template from which the PDMS flowcells were cast. The darkened features of the mask serve to block the exposure light leaving the raised features after solvent rinsing. These features become impressions in the PDMS during replica molding.

An additional step was taken to produce a higher quality access point for the laser directed into the flowcell. A small fused silica window (2 mm × 3 mm × 0.5 mm) was cut and placed, polished edge down, into the master mold, at laser beam insertion point

530 in Figures 5a and 5b, before the PDMS was added. Upon curing, the window was sealed in the PDMS with only small layers of PDMS on either side of the window, at the channel aperture. The small layers on the window in front of the channel aperture were cut away using a razor blade. This produces an air – glass – liquid interface for the laser beam path 560 shown in Figures 5a and 5b. The PDMS master was also designed to incorporate a smooth wall surrounding the entire system for visual access from the side walls 550 in Figure 5a. After using a razor blade to cut through the moat, cut shown as dashed line, e, in Figure 5a, the remaining exposed surface allowed for side observation through a cast rather than cut surface.

Figure 5b, shows the basic design of the PDMS flowcell from a side view. The fluid channels are shown as horizontal dashed lines, and the reservoirs as vertical lines. A glass cover slip 590, Figure 5b, was placed on the side of the flowcell that was at the bottom of the master mold as indicated in the illustration. Contact between the polished window edge and the glass cover slip was enhanced with a thin layer of PDMS cured between the channel replica and the final glass coverslip used to contain the channel structure. The top of the mold, as indicated in Figure 5b, had 1.5 mm diameter holes 580 cored to allow fluid access to the sealed fluidic network.

As shown in Figure 6, the PDMS flowcell was used in concert with a polycarbonate fluidic connection platform 600. The PDMS flowcell 610 is placed in contact (via the top of the PDMS mold) with the platform while aligning the reservoirs with the holes drilled into the platform, as in Figure 6a. This allows standard fittings 620,

630 to be used to connect the flowcell to the flow injector. The polycarbonate stock was drilled using a 0.025" diameter bit from the side and top meeting in the center to form an "L" shaped conduit, as in Figure 6b. The actual distances are 14mm in and 5 mm up to the surface resulting in a total swept volume of 6 μL for each conduit.

Optics and Fluidics

A diagram of the overall optical separation system can be seen in Figure 7. As can be seen in this diagram, the laser beam is directed to the focusing lens and ultimately into the flowcell using mirrors 710, 720. One mirror is mounted on the optical bench, while the other is mounted on a platform that is attached to the focusing mechanism of the microscope. This allows freedom in image focusing while maintaining the position of the beam within the flowcell. The optical separation system consists of an Argon ion laser 700 operating at 488 nm with a power of 0.2-1.0 W. The beam was focused using a 1" diameter 50 mm plano-convex BK7 focusing lens (730) into one of the flowcell designs described above. This lens (730) was mounted on a linear translation stage (740), allowing the position of the focal point to be changed along the retention distance axis. Moving the focal point provides micromanipulation capability in the separation dimension which can be used to selectively elute downstream particles. If the focal point is moved upstream from their position, this will cause them to be carried downstream by the liquid flow, now no longer retained by the focal point. The flowcell was held on a f-axis positioning stage (765) to facilitate alignment of the fluid pathway with the laser beam. The optical power used to calculate theoretical values was obtained from

measurements of the optical power of the laser beam after the focusing lens but outside the flowcell. Imaging was accomplished using a microscope 750 with a cooled CCD camera 755. Images were obtained with a 488 nm partially blocking filter using either a 5 × or 10 × objectives (760) for an overall image magnification of approximately 50 × or 100 × (particles were visualized in laser scatter). The minimum (downstream) and maximum (upstream) positions of the particle bands were calculated from high resolution image sequences collected with the CCD camera while the particles were trapped.

The flow system consisted of a syringe pump 770 connected to 0.02" i.d. PTFE tubing. Bead samples were introduced into the flow via an injector (775), fitted with a 2 μL injection loop. During separations, the volume flow rate was set at 2.0 μL/min and the linear velocity of the particles was calculated by measuring the distance traveled between video frames and the precisely known time from the recorded capture times.

Optical Separation Methods

The ability to optically separate particles based upon refractive index or size is strongly dependent on the methods of operating the instrument. Several steps must be taken during optical alignment, instrument setup and operation to yield acceptable results.

1. Laser beam must be aligned so that its height is centered on the flowcell aperture and passes through the center line of the microscope objective. In addition, the horizontal angle across the flowcell should not be too severe. Some deviation is tolerable as the flowcell can be aligned to the beam.

2. The beam quality and focal point are checked by using a low power and injecting a fluorescent solution (typically, Safranin O for a 488 nm – 515 nm Ar+ laser) and observing the beam. Adjustments can be made and the solution flushed from the flow cell.
3. Samples are pumped into the flowcell at a high volume flow rate (typically, 100 μL/min or more) to minimize the time between sample injections. As the particle band approaches the focal point and microscope field of view, the flow rate is reduced and the laser power temporarily increased to trap a sufficient number of particles.
4. Once the particles are trapped and the remaining particles in the injection have passed, the flow rate is further reduced causing the particles to move upstream. Once the band is moving upstream, the laser power is reduced causing the particles to move back downstream and separate into their equilibrium positions.
5. If a less dense band of trapped particles is desired, the beam can be alternatively blocked and unblocked by activating a flipper as necessary to thin the trapped particle population. Blocking the beam causes the downstream particles to flow past the focal point. When the beam is unblocked, those particles that are downstream of the focal point can no longer be trapped. This technique has been used to reduce a large group of particles to several or even a single individual. Additionally, the linear translator holding the focusing lens may be moved to elute downstream particles.

Chemicals for Analysis

The chemically different but uniformly sized spheres were obtained from Bangs Laboratories Inc. (Fisher, IN): 1.97 μm diameter polystyrene (PS) and 1.98 μm diameter polymethlymethacrylate (PMMA) were dispersed in distilled water with 2 % Tween-20 added to minimize aggregation. The mobile phase also contained 2% Tween-20 which resulted in a refractive index of $n_1 = 1.3353$, slightly above that of pure water ($n_1 = 1.3329$). The refractive indices of the beads as reported by the manufacturer are PS, $n_2 = 1.59$, and PMMA, $n_2 = 1.49$.

Results

The optical separation results were demonstrated using the glass flowcell, and the other designs tested using a fluorescent dye in the flowcell to ascertain the quality of the beam focus.

As shown in Figure 8, the beam focal point 800, using a 50mm focal length lens, can clearly be seen in the image when a fluorescent solution is placed in the flowcell. From this data the beam radius and all distances from the focal point, Z, were calculated.

In Figure 9a, the laser beam first traverses the region of air 920 created by the moat design of the PDMS master. It then reaches the focal point 900 after being focused directly into the flowcell containing water through the PDMS wall 910. As can be seen, there is significant distortion of the beam which results in little or no optical separation / trapping ability. In Figure 9b, the laser beam focal point 950 was focused into a PDMS

flowcell containing water with the incorporated glass window 940, bordered on the other side by air 950. Using the PDMS flowcell with the optical window incorporated resulted in no such beam distortion as seen in Figure 9b. The beam and focal point was imaged using a fluorescent solution of safranin O. Channel widths are 750 μm.

To demonstrate the optical separation capabilities, pure solutions of PS and PMMA were injected sequentially into the optical separation system using a glass flowcell, and finally a mixture of the two beads was injected. The results are summarized as follows: The higher refractive index particles (PS, n = 1.59, greater scattering) were stopped in the liquid flow at a point farther from the focal point (upstream) and the lower refractive index particles (PMMA, n = 1.49, lower scattering) were retained closer to the focal point (downstream). The position of the particles when injected individually and as a mixture can be seen in Figures 10a, 10b, and 10c corresponding to pure PMMA, pure PS, and a mixture of PS and PMMA respectively. In this image much of the scattered 488 nm light was removed by a filter, but small residual quantities allow the particles to be visualized. For each of the injections, many (> 10) particles were stably trapped; they remained trapped for the duration of the experiments which ranged from 5 min - 20 min in a stable, balanced equilibrium state. The average positions of the bands (average of the minimum and maximum positions) during each experiment are compared with the theoretically calculated values and given in Table 1. The experimental values agree well with those obtain from theory as given by % errors between 4 % and 14 %. The separation positions are also summarized graphically in Figure 11. The inter-band

distance, or separation distance, was 0.66 mm when comparing pure injections and 0.52 mm for the mixture injection.

Table 1. Comparison of Theoretical and Mean Experimental Optical Separation Retention Distances, Z

	Mean Experimental		Theoretical	Theory
Sample	**Mean Z**	**σ (mm)**	**Z (mm)**	**% Error**
PMMA, mixture (N=7)	1.71	0.20	1.78	3.7
PMMA, pure (N=6)	1.71	0.12	1.57	-8.7
PS, mixture (N=6)	2.23	0.30	2.60	14.2
PS, pure (N=6)	2.37	0.21	2.72	12.8

~~D.~~